

Pharmacologic Approaches to Protection against Radiation-induced Lethality and Other Damage

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Studies on mechanisms of radioprotection are leading to a more rational use of protectors for different applications. In considering the feasibility of radioprotectors that act through various mechanisms, it is necessary to distinguish the application needed, e.g., protection against accidental external or internal exposures; acute high-dose radiation injury or low doses over a long period, high-LET radiation exposures during space flight, and protection of normal tissues of cancer patients who are undergoing therapy. Protectors generally are classified as either sulfhydryl compounds, other antioxidants, or receptor-mediated agents (e.g., bioactive lipids, cytokines, and growth factors). This review focuses on comparative radioprotection and toxicity studies in mice using the most effective phosphorothioate agents designated as WR-compounds and other classes of protectors. The superiority of phosphorothioates (WR-2721, WR-151327) as radioprotectors appears to be related to their high affinity for DNA and the similarity in structure of phosphorothioate metabolites to polyamines, and their effects on processes related to DNA structure and synthesis. Drug tolerance levels are available from clinical trials using WR-2721 (amifostine) and provide a basis for discussions of the disadvantages of phosphorothioate administration outside a clinical setting. In this regard, arguments are presented against the current use of WR-2721 by Department of Energy personnel for planned radiation exposures during emergencies. Future research may demonstrate, however, that pharmacologic agents could be useful in accident scenarios, especially when used in combination with therapeutic measures. Assessment of potential prophylactic measures should consider compatibility with therapeutic measures currently in use or ones that might be available in the future for the treatment of radiation injuries. These include antiemetics, purified stem cells, granulocyte colony-stimulating factor, and other cytokines. Their potential usefulness against radiation-induced mutagenesis of pre- and postexposure administration of phosphorothioates and other classes of protectors should be corroborated in humans. — *Environ Health Perspect* 105(Suppl 6):1473–1478 (1997)

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Development of Radioprotectors

The first *in vivo* studies on protection by chemicals against ionizing radiation were conducted almost 50 years ago. Patt

reported in 1949 that cysteine, a sulfur-containing amino acid, could protect rats from a lethal dose of X-rays (1). With the nuclear age already a reality, potential military applications of radioprotective

chemicals and their use in the event of nuclear accidents appeared to be a distinct possibility. From the earliest days of research, it was also hypothesized that cancer therapy could be improved by the use of radioprotectors to protect normal tissue from radiation damage. As research on radioprotectors developed, it became evident that studies with these chemicals could provide important information on mechanisms of interaction of radiation and biomolecules. In the 1950s through a program supported by the Atomic Energy Commission, aminoethylisothiourea was developed and understanding of sulfur-containing radioprotectors increased.

From 1959 until 1973, the Walter Reed Army Institute of Research (WRAIR) supported an antiradiation drug development program, in which more than 4000 compounds were synthesized and screened in mice (2). From 1979 to 1988, the WRAIR program was reinstated and its most significant contribution was development of amifostine (WR-2721) and related phosphorothioates (3). Phosphorylated compounds serve as pro-drugs for the active free aminothiols. During this period, WR-2721 was introduced into cancer clinical trials to study protection against normal tissue damage caused by radiotherapy and various chemotherapeutic agents (4,5). To date, the U.S. military has not approved for use or further development WR-2721 or any agent that might protect against radiation-induced lethality. Related research has been carried out in the past in the former Soviet Union (6) and other Warsaw Pact countries. A thorough review and comparison of the many drugs studied under the competing programs have not yet been done.

Although most older programs, including one at the National Institutes of Health from 1980 to 1983 (7), emphasized drug screening in experimental animals, during recent years studies have emphasized a shift toward biological mechanisms and agents. During the 1980s until the present, there have been a variety of diverse smaller research programs characterized by the ascendancy of biotechnology and new knowledge related to cellular radiation effects, e.g., cellular sensitivity, cell cycle, and cell death. Current research is also leading to an increased understanding of the molecular mechanisms of protection by WR-compounds (8–10). Certain chemical and biological protectors may

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Abbreviations used: DMF, dose-modifying factor; G-CSF, granulocyte colony-stimulating factor; GI, gastrointestinal; GM-CSF, granulocyte-macrophage colony-stimulating factor; MPG, 2-mercaptopyropionylglycine; MTD, maximum tolerated dose; WR-compounds, numerical designations of Walter Reed Army Institute of Research; WRAIR, Walter Reed Army Institute of Research; WR-1065, 2-(3-aminopropylamino)ethanethiol; WR-2721, Ethiol, amifostine, ethiofos, gammaphos, S-2-(3-aminopropylamino)ethylphosphorothioic acid; WR-3689, S-2-(3-methylaminopropylamino)ethylphosphorothioic acid; WR-151326, 3-(3-methylaminopropylamino)propanethiol; WR-151327, S-3-(3-methylaminopropylamino)propylphosphorothioic acid.

lead to the same result, because different biological end points (cell transformation, loss of proliferative capacity, apoptotic death, etc.) may arise from radiation damage at different cell targets. Therefore, clear differences between chemical and biological mechanisms are not always evident. The information evolving from the larger genome project predictably will lead to better radioprotectors through improved understanding of DNA structure, function, and repair, radiation-induced cancer, and identification of radiation-sensitive individuals. With the successful development of products by the biotechnology industry, there is also a shift in emphasis from radioprotectors that need to be administered before radiation exposure to treatments, such as cell growth factors, that can result in increased survival. Granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been recommended for use in treatment of accidental radiation injuries (11,12). A number of reviews and books on radioprotection have been published that include discussions from diverse viewpoints of classes of protectors and their mechanisms of action (13–21).

Comparison of Efficacy and Toxicity of Radioprotective Agents

The most widely used and preferred procedure for comparing the efficacy of protective agents in experimental animals has been to determine the dose-reduction factor or dose-modifying factor (DMF). DMFs have been obtained by irradiating mice with and without administered agents at a range of exposures. The DMF for 30-day survival quantifies protection of the hematopoietic system (7,22). With the loss of hematopoietic stem cells, death follows from infection, hemorrhage, and anemia. Some protection against the hematopoietic syndrome, a result of damage to bone marrow stem cells, has been shown in rodents using a variety of agents that provide protection, repair, or regeneration. These include the many agents discussed in the reviews cited above, such agents as: thiols, other antioxidants, vitamins, enzymes and synthetic compounds that have enzymatic activity, nitroxides, nitrones, immunomodulators, endotoxin derivatives, growth factors, xanthine and adenosine derivatives, eicosanoids, calcium antagonists, and polyamines. With the availability of radioprotective agents and biological factors for treatment of the hematopoietic syndrome,

there is a renewed interest in how to protect against the gastrointestinal (GI) syndrome (23). GI death generally is assessed by determining survival at 6 or 7 days after comparatively high doses of whole-body irradiation (7,22,23).

The most useful preclinical studies relate protective effects to toxicity in the same animal model. For example, drugs are administered at one-fourth or one-half the maximum tolerated dose (MTD), where the MTD is approximately equal to the dose that causes death in 10% of the mice of a specific strain (7). Also useful in comparing agents is determination of protection based on doses that cause other measurable toxicities such as behavioral toxicity or performance decrement (24,25). Automated monitoring of spontaneous locomotor activity is one of several approaches for estimating behavioral toxicity in irradiated and/or drug-treated mice (26,27).

Phosphorothioates

Early studies have reported very large DMFs in mice treated with WR-2721 and other phosphorothioates, but the drug doses used were unreasonably high (22). Nevertheless, the greatest protection observed in experimental animals has been obtained with phosphorothioates such as WR-2721, WR-3689, and WR-151327 (7), with the possible exception of agents that induce hypoxia as their main mode of action. The time of administration relative to radiation exposure is critical and the efficacy of the compounds is strongly related to pharmacokinetic considerations as well as to radiochemical considerations. For example, oral administration of WR-2721 is compromised not only by hydrolysis in the stomach but also because of first-pass metabolism, which probably has components of both gut wall and liver metabolism (28). Intraperitoneal administration at one-half MTD before mice are exposed to γ -irradiation results in a DMF for 30-day survival of 2.0 to 2.2 for WR-2721, WR-3689, or WR-151327 (7,29,30). At a more realistic dose of one-fourth MTD (e.g., 200 mg/kg for WR-2721), the DMF is 1.6 to 1.8 for any of the three drugs (29–34). At one-fourth MTD, decreases in locomotor activity are still observed. The decrease in locomotor activity caused by phosphorothioates may be due to diverse pharmacologic mechanisms, including hypotension (35), not to hypothermia. The decrease in locomotor activity can be mitigated by diverse agents (e.g., caffeine, theophylline, propranolol), while these

agents either augment or do not affect radioprotection by WR-2721 (26,30,32–34). The behavioral toxicity of phosphorothioates has been shown by other assays in different species, including decrements in learned tasks (36–38).

Phosphorothioates administered *ip* provide substantial protection against acute intestinal radiation injury; the DMF for GI death after treatment with most phosphorothioates is 0.7 to 0.8 the DMF for hematopoietic death (23). WR-2721 and WR-151327 are also effective against neutron exposure (39). When phosphorothioates are administered orally to mice, protection against lethality from γ -radiation exposure decreases compared to survival after *ip* or intramuscular administration. The DMF for protection against 30-day lethality for oral administration of WR-2721, WR-3689, or WR-151327 at one-half MTD is 1.3 to 1.5 and at one-fourth MTD is 1.2 to 1.3 (29,40, unpublished data). WR-151327 is significantly less toxic when administered orally than is WR-2721 or WR-3689. However, it is not a better oral radioprotector than WR-2721 or WR-3689, because more of the drug must be administered to mice to obtain the same level of protection.

WR-2721 and WR-151327 protect against radiation-induced malignancies in rodents when they are administered before radiation exposure (41–43). It is not clear whether phosphorothioates have an anticarcinogenic effect when administered postirradiation. As early as 1967, genomic stabilization was proposed as a radioprotective mechanism for aminothiols (44). More recently, emphasis has been placed on how these agents might affect DNA repair processes (45,46). Grdina and co-workers have effectively demonstrated how WR-compounds influence post-irradiation processes including antimutagenic effects (47,48). The structural similarity of the metabolites of WR-compounds (e.g., WR-1065, WR-151326, and their disulfides) to endogenous polyamines suggests that they may interact with DNA similarly and that they may influence DNA protection, repair, and synthetic processes (8–10,34,48).

Other Sulfhydryl Compounds and Antioxidants

Many other sulfur-containing compounds as well as synthetic antioxidants with nonsulfhydryl moieties have been studied for their radioprotective effect. Maximum protection measured by 30-day lethality

generally is lower than that afforded by phosphorothioates. Because of the low toxicity and substantial history of human use for some of these agents, for example, *N*-acetylcysteine, diethyldithiocarbamate, 2-mercaptopropionylglycine (MPG), nimodipine, propranolol (16,32,49,50), further studies in specific exposure situations are warranted. The phosphorothioates are effective against lethality only when they are administered before radiation exposure and the time window for protection is short. Several other thiols, however, have been reported to provide some protection when administered after radiation exposure, e.g., MPG (50). Some naturally occurring radioprotectors, e.g., vitamins E, A, and C, superoxide dismutase, minerals that mimic or induce activity of endogenous antioxidant systems (16), protect only against lower doses of radiation (DMF of 1.1–1.2) but have low toxicity, possible increased benefits from longer use, and a wider window of protection. The lower degree of protection by these agents compared to those of synthetic agents may be related to modulation of later reactions, for example interaction of radiation-induced radicals of biomolecules with reactive oxygen species evolved during normal cellular processes. Protection against lethality has also been reported for postirradiation administration of natural antioxidants such as superoxide dismutase, selenomethionine, vitamin A, and vitamin E (16,31,51–53). A number of synthetic and natural antioxidants such as vitamin E, superoxide dismutase, MPG, and ginkgo biloba exhibit antimutagenic properties when administered after radiation exposure (50,53–55); other studies also suggest that antioxidant vitamins and minerals can modulate radiation-induced carcinogenesis (56,57). If ongoing chemoprevention trials (58) show that antioxidants (vitamin E, β -carotene, selenium compounds) have a general protective effect against cancer development, it appears reasonable to assume that they would be effective against radiation-induced cancer in humans.

Receptor-mediated Protectors

Identification of specific receptors for many radioprotectors will provide a greater understanding of the mechanisms of action of radioprotective agents at the cellular level. This diverse class of radioprotective agents, which has known receptors, has many subclasses and includes bioactive lipids, naturally occurring peptides, and some synthetic compounds (30). Of the

compounds acting through receptor mediation, the most protective appear to be natural and synthetic eicosanoids (59). Protection against lethality, in general, is slightly lower than that afforded by phosphorothioates, and like phosphorothioates, eicosanoids are effective only when administered before radiation exposure. The prostaglandin analog misoprostol appears to provide relatively greater protection against GI injury than against hematopoietic injury (23). Although the mechanism of action of misoprostol is probably very different from that of phosphorothioates, it also has been reported to protect against radiation-induced oncogenic transformation (60). Eicosanoids and other biological protectors exhibit behavioral toxicity at least as great as that of chemical protectors such as phosphorothioates (26), therefore relative to toxicity, protection generally is lower. The use of eicosanoids for radioprotection outside a clinical setting probably will be hampered by their extensive physiologic effects at low doses, for example, misoprostol has strong abortifacient properties. However, the use of bioactive lipids as protectors (or inhibition of their synthesis) in the setting of cancer treatment may have promise because of exploitable differences in eicosanoid metabolism between normal and tumor tissue.

Studies on protection by immunomodulators date from reports indicating that endotoxin provides protection when administered 20 to 24 hr before irradiation and shortly before or after exposure (61). In general, immunotherapeutic agents, cytokines, or growth factors provide a large window of protection, although protection against lethality is lower (DMF = 1.2–1.3) than that afforded by phosphorothioates. Administration of cytokines and growth factors after radiation exposure combined with preirradiation administration of phosphorothioates appears synergistically to reduce radiation damage (30,62). The possible mechanisms of protection by cytokines have been reviewed by Neta et al. and Neta (63,64). The potential utility of cytokines and growth factors as therapeutic agents and/or protective agents is great, and future studies should lead to specific agents to treat specific tissue damage (12).

Applications of Radioprotectors in Various Scenarios

In considering the feasibility of radioprotectors, it is necessary to distinguish

the application desired. For example, uncontrolled exposure to radiation from nuclear weapons, space, or accidents presents challenges unlike those encountered in radiotherapy. The requirements for protecting an astronaut against space radiation would be different taking into consideration physiological complications posed by microgravity and potential exposures to high-LET radiation. Lessons learned from accidents have shown that benefits can be gained by treatments at different stages in relation to various types of radiation exposure: blockers (potassium iodide), chelating agents, internal decontaminating agents (Prussian Blue), supportive therapy (platelets, fluids, antibiotics), colony-stimulating factors (6,11,12,65).

The problem of toxicity of protectors is more acute when the intended use is a situation in which performance is an important factor. Nausea and vomiting occur in most patients treated with WR-2721 (4), and use of effective antiemetics is recommended (66). Hypotension is a dose-limiting side effect in patients, and the side effects observed in rodents treated with WR-2721 may be related to this property (35). The military requirement for an agent to be given before radiation exposure emphasizes performance and preference for a radioprotector that is effective against the detrimental effects on performance of high-dose radiation. Consequently, a NATO panel has recommended the use of granisetron, an antiemetic, which is, in effect, a behavioral radioprotector. As a 5-hydroxytryptamine-3 receptor antagonist, granisetron ameliorates radiation-induced emesis (67).

Proposed Use of WR-2721 by Department of Energy Personnel

A consensus conference was held on 15 and 16 August 1996 subsequent to a proposal submitted by U.S. Bioscience Corporation for the use of Ethylol (amifostine, WR-2721) in planned radiation exposures during emergencies. The conference was held to produce a document (68) to advise the Secretary of Energy on the proposed use of the drug according to the protocol developed by U.S. Bioscience. Sixteen attendees approved the statement and four attendees declined to sign the recommendation. The consensus group recommended against current application of the drug to U.S. Department of Energy operations and against its prospective use in radiation accident response (68). Any consideration for use of Ethylol in future radiation accident situations would require

extensive research and testing demonstrating clear benefit to emergency workers.

The group's recommendations (68) were based on the following conclusions. *a)* The drug is not currently approved for such use by the U.S. Food and Drug Administration. *b)* Intravenous administration is problematic (15-min infusion with preadministration of antiemetic; blood pressure monitored during drug administration) and other routes and formulations require further development and evaluation by the corporation. *c)* Most emergency situations require prompt action and there are time limitations associated with the use of the drug. *d)* There remains uncertainty regarding potential side-effects, particularly considering the conditions of extreme physical and psychological stress found in emergencies (including use of protective gear).

e) The emergency circumstances when use of the drug might be considered have not been defined (potential hazards from U.S. Department of Energy operations involve internal deposition and protracted exposures to radionuclides).

At present there is a lack of quantitative data from clinical studies on phosphorothioates or any other protective agent to perform a proper risk-benefit analysis with respect to their use in emergency scenarios. Efficacies of suggested doses should be quantified using measures or biological markers that allow determination of a DMF that reflects protection against radiation, e.g., bone marrow protection. Although WR-2721 protects irradiated patients from hematologic toxicity as measured by white blood cell and platelet count depression (69,70), phase III trials are needed for more

definitive data. It is unlikely that the tolerable dose of WR-2721 (740–900 mg/m²) would provide a DMF for protection against lethality because of bone marrow damage greater than 1.2 based on estimates from pharmacokinetic studies in different species (71). Comparative analyses of outcomes derived from preirradiation protector administration versus treatments, for example, with G-CSF or GM-CSF, are desirable. Possible antimutagenic effects of WR-compounds (47,48) and other agents administered either before or after radiation exposure need to be confirmed and quantified within ongoing clinical trials, e.g., by assessing mutations at the *hprt* locus. Similarly, the potential anticarcinogenic effects of various agents could be tested in studies in patients on secondary tumor induction attributable to radiation treatment.

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